CLAIMS

What is claimed is:

1. A method to calibrate a multi-channel fluorescent polynucleotide separation apparatus, comprising:

introducing a fluorescent polynucleotide separation standard into a channel of said apparatus, wherein the standard comprises at least two polynucleotides of different length, each of the polynucleotides being labeled with a spectrally distinct fluorescent dye,

separating the polynucleotides from each other,

collecting spectra information of the dye standards,

establishing pure dye spectra and multi-component values for the dyes;

generating spectra data files based on the established pure dye spectra and multi-component values, and

storing the spectra data files on a computer system adapted for communication with the apparatus for use in sample analysis.

2. The method of claim 1, wherein the standard comprises at least four polynucleotides of different length, each of the polynucleotides being labeled with a spectrally distinct fluorescent dye.

3. A method to estimate reference spectral profiles of selected fluorescent dyes using a fluorescent polynucleotide separation apparatus, said method comprising the steps,

introducing a fluorescent polynucleotide separation standard into said apparatus, wherein the standard comprises at least two polynucleotides of different length, each of the polynucleotides being labeled with a spectrally distinct fluorescent dye,

separating the polynucleotides from each other,

detecting the separated polynucleotides with a detector, wherein the detector collects spectral data from the separated polynucleotides over a plurality of spectral channels, and collects temporal data from the separated polynucleotides over a plurality of temporal points, and

generating a total emission temporal profile from the spectral and temporal data.

4. The method according to claim 3, further comprising the step of detecting peaks in the total emission temporal profile.

5. The method according to claim 4, further comprising the step of selecting a reference spectrum for each of the fluorescent dyes, wherein each reference spectrum substantially corresponds to a detected peak of the emission temporal profile.

6. The method of claim 5, wherein each reference spectrum is corrected by estimating the net analytical signal for each spectral channel.

7. A system to estimate reference spectral profiles of selected fluorescent dyes using a fluorescent polynucleotide separation apparatus, said system comprising a processor and a computer readable medium functionally coupled to said processor for storing a computer program, comprising:

computer code that receives plurality of spectral and temporal data from a fluorescent polynucleotide separation apparatus, and

computer code that calculates a total emission temporal profile from the spectral and temporal data.

8. A calibration standard for a fluorescent polynucleotide separation apparatus, the standard comprising: at least four polynucleotides of different length, each polynucleotide labeled with a different fluorescent dye having a distinctive spectral profile having a peak, wherein the lengths of the polynucleotides differ from one another such that, upon electrophoretic separation, the peak of the spectral profile of any one of the dyes does not significantly overlap the peak of the spectral profile of any of the other dyes.

9. The calibration standard of claim 8, wherein the fluorescent labeled polynucleotides in the standard are separated by at least 10 bases in length.

10. A method of monitoring a separation channel of a fluorescent polynucleotide separation apparatus, said method comprising:

(i) introducing a fluorescently labeled polynucleotide composition to an inlet end of separation channel of a fluorescent polynucleotide separation apparatus, said composition comprising (a) a polynucleotide labeled with a first fluorescent dye, and (b) a monitoring dye that is spectrally distinct from the first fluorescent dye;

(ii) causing the composition to migrate down the channel; and

(iii) detecting for the monitoring dye at one or more regions downstream of said inlet end;

whereby detection of the monitoring dye at said one or more regions is indicative of flow along the channel.

11. The method according to claim 10, wherein the composition comprises

a plurality of polynucleotides labeled with at least two spectrally distinct fluorescent dyes, wherein the monitoring dye is spectrally distinct from each of the at least two spectrally distinct fluorescent dyes.

12. The method of claim 11, wherein the polynucleotides labeled with at least two spectrally distinct florescent dyes is a polynucleotide sequencing reaction product mixture.

13. The method of claim 12, wherein the monitoring dye is attached to a polynucleotide.

14. A composition for monitoring flow of electrical current through a separation channel of a fluorescent polynucleotide separation apparatus, said composition comprising:

(a) a polynucleotide sample comprising a plurality of polynucleotides of unknown nucleotide sequence, each being associated with a first, second, third or fourth fluorescent dye, with each of said first, second, third, and fourth fluorescent dyes being present in said sample; and

(b) a monitoring standard comprising a polynucleotide associated with a fifth fluorescent dye, said fifth fluorescent dye being spectrally distinct from each of the first, second, third and fourth fluorescent dyes.

15. The composition according to claim 14, wherein the fluorescent dye labeled polynucleotide sample is a polynucleotide sequencing reaction product mixture.

16. A method to calibrate a multi-channel fluorescent polynucleotide separation apparatus having one or more spectral sensors, comprising:

introducing a fluorescent polynucleotide separation standard into a channel of said apparatus, wherein the standard comprises at least two polynucleotides of different length, each of the polynucleotides being labeled with a spectrally distinct fluorescent dye,

separating the polynucleotides from each other,

collecting spectra information for each of the separated dyes at each of the sensors,

estimating a sensitivity value for each of the dyes at each of the sensors.

17. The method of claim 16, wherein the standard comprises at least four polynucleotides of different length, each of the polynucleotides being labeled with a spectrally distinct fluorescent dye.

18. The method of claim 16, further comprising generating a calibration matrix comprised of the sensitivity value estimates.